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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/495 — A1

(11) International Publication Number: **WO 97/31637**

(43) International Publication Date: 4 September 1997 (04.09.97)

(21) International Application Number: PCT/EP97/00897

(22) International Filing Date: 25 February 1997 (25.02.97)

(30) Priority Data:
MI96A000378 28 February 1996 (28.02.96) IT

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: USE OF 5-HT$_{1A}$ RECEPTOR ANTAGONISTS FOR THE TREATMENT OF URINARY INCONTINENCE

(57) Abstract

Compounds which: (a) bind to a 5-HT$_{1A}$ receptor with an affinity of at least 10^{-7}M, (b) bind to a 5-HT$_{1A}$ receptor with an affinity at least 50-fold stronger than the affinity with which the compound binds to an α_1-adrenergic receptor, and (c) exhibit 5-HT$_{1A}$ receptor antagonist activity on both pre-synaptic and post-synaptic 5-HT$_{1A}$ receptors, and their stereoisomers, hydrates, solvates and pharmaceutically acceptable salts are useful for the treatment of lower urinary tract disorders in mammals.

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TITLE

Use of 5-HT_{1A} Receptor Antagonists for the Treatment of Urinary Incontinence

DESCRIPTION

The invention relates to compositions and methods for treating certain disorders of the lower urinary tract in mammals, including humans, using serotonin 5-HT_{1A} receptor antagonist compounds which exert their inhibitory effects via both pre-synaptic (somatodendritic) and post-synaptic antagonism.

In mammals, micturition is a complex process which requires the integrated actions of the bladder, its internal and external sphincters, the musculature of the pelvic floor, and neurological control over these muscles at three levels (in the bladder wall or sphincter itself, in the autonomic centres of the spinal cord, and in the central nervous system at the level of the pontine micturition centre in the brainstem (pons) under the control of the cerebral cortex). Micturition results from contraction of the detrusor muscle, which consists of interlacing smooth muscle fibres under parasympathetic autonomic control from the sacral spinal cord. A simple voiding reflex is formed by sensory nerves for pain, temperature, and distension that run from the bladder to the sacral cord. However, sensory tracts from the bladder also reach the pontine micturition centre, resulting in the generation of nerve impulses that normally suppress the sacral spinal reflex arc controlling bladder emptying. As a result, normal micturition is initiated by voluntary suppression of cortical inhibition of the reflex arc and by relaxation of the muscles of the pelvic floor and the external sphincter. Finally, the detrusor muscle contracts and voiding occurs.

Functional abnormalities of the lower urinary tract, e.g., dysuria, incontinence, and enuresis, are common in the general population. Dysuria includes urinary frequency, nocturia, and urgency, and may be caused by cystitis, prostatitis or benign prostatic hypertrophy (which affects about 70% of elderly males), or by neurological disorders. Incontinence syndromes include stress incontinence, urgency incontinence, and overflow incontinence. Enuresis refers to the involuntary passage of urine at night or during sleep.

Prior to the present invention, treatment of neuromuscular disorders or the lower urinary tract has involved administration of compounds which act directly on the bladder muscles, such as flavoxate, a spasmolytic drug also active on the pontine micturition centre, or anticholinergic compounds such as oxybutynin. The use of α_1-adrenergic receptor antagonists for the treatment of benign prostatic hypertrophy is

also common. However, treatments that involve direct inhibition of the pelvic musculature (including the detrusor muscle) may have unwanted side effects such as incomplete voiding or accommodation reflex paralysis, tachycardia and dry mouth. Thus, it would be preferable to utilize compounds which act via the peripheral or central nervous system, for example to affect the sacral spinal reflex arc and/or the inhibition pathways of the pontine micturition centre in a manner that restores normal functioning of the micturition mechanism.

Lecci et al (*J. Pharmacol. Exp. Therapeutics*, 262, 181, 1992) describe the effects of the 5-HT_{1A} receptor ligands 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT) and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)-butyl]-piperazine (NAN-190) on micturition reflexes in the anaesthetized rat. 8-OH-DPAT (an agonist) stimulated the supraspinal micturition reflex originating from the pontine micturition centre, while NAN-190 inhibited the supraspinal micturition reflex. The authors concluded that spinal and supraspinal 5-HT_{1A} receptors modulate the supraspinal micturition reflex in this system. The present inventors, however, have found that the efficacy of NAN-190 and other 5-HT_{1A} receptor ligands in inhibiting the supraspinal micturition reflex is directly correlated to their relative binding affinities for the α_1-adrenergic receptor, rather than to their affinities, if any, for the 5-HT_{1A} receptor which called into question the relevance of the effects correlated to 5-HT_{1A} receptor activity for lower urinary tract disorders. Finally, as discussed below, NAN-190 is considered a partial 5-HT_{1A} receptor agonist rather than a complete or "true" antagonist. Therefore, prior to the present invention, the use of true 5-HT_{1A} receptor antagonists to treat urinary tract disorders was unknown.

At least two functionally distinct types of the 5-HT_{1A} receptor have been identified, and these are designated "pre-synaptic" (or somatodendritic) and "post-synaptic". Pre-synaptic receptors are present on 5-HT-producing neurons and are involved in autoregulation of 5-HT release; their activation causes physiological changes including hyperphagia, hypothermia (in the mouse), bradycardia and hypotension. Post-synaptic receptors are widely distributed throughout the mammalian brain and are coupled to potassium channels and adenylate cyclase; their activation leads to "5-HT behavioural syndrome", hypothermia (in the rat), and elevation of plasma corticotropin levels. Beyond the differences in their anatomical distribution and functioning, pre-synaptic and post-synaptic receptors can be distinguished by the differential activity profiles of different 5-HT_{1A} receptor ligands. For example, full agonists such as 8-OH-DPAT and 5-carboxytryptamine have agonist activity on both pre-synaptic and post-synaptic receptors. By contrast, partial agonists such as buspirone, ipsapirone, spiroxantine,

urapidil, NAN-190 and BMY 7378 have agonist activity on pre-synaptic receptors and antagonist activity on post-synaptic receptors. Finally, some compounds exhibit antagonistic activity on both pre-synaptic and post-synaptic receptors. These last compounds, true antagonists of the serotoninergic 5-HT_{1A} receptor, are useful in the invention.

The invention provides the use of a compound which

(a) binds to a 5-HT_{1A} receptor with an affinity of at least 10^{-7} M,

(b) binds to a 5-HT_{1A} receptor with an affinity at least 50 times greater than the affinity with which the compound binds to an α_1-adrenergic receptor, and

(c) exhibits 5-HT_{1A} receptor antagonist activity on both pre-synaptic and post-synaptic 5-HT_{1A} receptors,

or of a stereoisomer, hydrate, solvate or pharmaceutically acceptable salt of such a compound, for the preparation of a medicament for the treatment of neuromuscular disorders of the lower urinary tract in mammals.

Compounds useful in the practice of the invention preferably bind to 5-HT_{1A} receptors with an affinity (K_i) of at least 10^{-8} M. Expressing their 5-HT_{1A} receptor antagonist activity (at both pre-synaptic and post-synaptic sites) as a function of dose, the compounds may have ID_{50} values of from 1 to 2000 μg/Kg, and preferably of from 1 to 200 μg/Kg.

As the compounds useful in the practice of the invention bind to 5-HT_{1A} receptors with an affinity at least 50 times greater, and preferably 100 times greater, than they bind to α_1-adrenergic receptors, they have α_1-adrenergic receptor binding constants in the micromolar range or weaker.

Medicaments prepared according to the invention are suitable for the treatment of neuromuscular disorders of the lower urinary tract, particularly those involving micturition, such as dysuria, incontinence, and enuresis. Without wishing to be bound by theory, it is believed that administration of 5-HT_{1A} receptor antagonists prevents unwanted activity of the sacral reflex arc and/or cortical mechanisms that control micturition. Thus it is contemplated that a wide range of lower urinary tract disorders can be treated using the compounds of the invention.

The compounds of the present invention may be formulated into liquid dosage forms with a physiologically acceptable carrier, such as phosphate buffered saline or deionized water. The pharmaceutical formulation may also contain excipients,

including preservatives and stabilizers, which are well-known in the art. The compounds can be formed into solid oral or non-oral dosage units such as tablets, capsules, powders, and suppositories, and may additionally include excipients, including without limitation lubricants, plasticizers, colorants, absorption enhancers, bactericides, and the like. Administration of medicaments prepared using true 5-HT_{1A} receptor antagonist compounds, their stereoisomers, pharmaceutically acceptable salts, hydrates or solvates, may be achieved by any effective route, including oral, enteral, intravenous, intramuscular, subcutaneous, transdermal, transmucosal (including rectal and buccal), and inhalation routes. Preferably, an oral or transdermal route is used (i.e., via solid or liquid oral formulations, or skin patches, respectively).

An effective amount of the medicament is an amount that results in measurable amelioration of at least one symptom of the disorder. This effective amount can be determined by experimentation known in the art, such as by establishing a matrix of dosages and frequencies and comparing a group of experimental units or subjects to each point in the matrix. Symptoms of urinary tract disorders include urgency, frequency, urine leakage, enuresis, dysuria, hesitancy, difficulty in emptying bladder. A measurable amelioration of any symptom or parameter is determined by a physician skilled in the art or reported by the patient to the physician.

For example, a single patient may suffer from several symptoms of dysuria simultaneously, such as, for example, urgency and frequency, either or both of which may be reduced using the methods of the present invention. In the case of incontinence, any reduction in the frequency or volume of unwanted passage of urine is considered a beneficial effect of the present methods of treatment.

The amount of the agent to be administered may range from about 0.01 to about 25 mg/kg/day, preferably from about 0.1 to 10 mg/kg/day and most preferably from about 0.2-5 mg/kg/day. It will be understood that the medicament formulations of the present invention need not in themselves contain the entire amount of the agent that is effective in treating the disorder, as such effective amounts can be reached by administration of a plurality of doses.

In a preferred embodiment of the invention, N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]--ethyl}-N-(2-pyridyl)-cyclohexanecarboxamide (hereinafter Compound A) is formulated in capsules or tablets each containing from 50 to 200 mg of Compound A, and is administered to a patient at a daily dose of 200 mg for relief of urinary incontinence.

In another preferred embodiment of the invention, N-{2-[4-(4-indolyl)-1-piperazinyl]--ethyl}-N-(2-pyridyl)-cyclohexanecarboxamide (hereinafter Compound B) is formulated in capsules or tablets each containing from 20 to 60 mg of Compound B, and is administered to a patient at a daily dose of 60 mg for relief of urinary incontinence.

Compounds which possess the requisite pre-synaptic and post-synaptic 5-HT_{1A} antagonistic activity may be found amongst those having a protonatable nitrogen atom which is linked on one side to an aromatic or heteroaromatic ring and to a carbon chain on the other side. In addition, the protonatable nitrogen and the chain can form a ring. Compounds belonging to this general class may be tested according to the methods discussed below to determine whether they meet the other criteria for use in the invention.

Measurement of the specific binding activity of a compound towards different neuronal receptors (such as serotoninergic 5-HT_{1A} receptors, α_1- or α_2-adrenergic receptors, dopaminergic D_2 receptors, and serotoninergic 5-HT_2 receptors) may be achieved using any of a multiplicity of methods that are well known in the art, such as competitive binding to native or cloned receptors. Typically, a biological source of, for example, a 5-HT_{1A} receptor is used in which the receptor is present at a sufficiently high concentration so that labelled 5-HT or a labelled 5-HT_{1A} ligand is easily measurable. This source may comprise a mammalian tissue or fluid (either in situ or after removal) or a tissue culture cell. The target receptor may be expressed from either an endogenous gene or from a transfected receptor-encoding recombinant gene. For example, the rat hippocampus is a rich source of 5-HT_{1A} receptors. Alternatively, human 5-HT_{1A} receptor cDNA can be expressed in *E. coli* cells in culture as reported in Bertin B. et al., *J. Biol. Chem.*, 267, 8200 (1992). The ability of the test compound to compete with labelled 5-HT (or a labelled 5-HT_{1A} ligand) for receptor binding is then measured, and a binding constant is calculated using Scatchard analysis or equivalent computational methods well known in the art.

It will be understood that measurements of receptor binding affinity of a particular compound may vary depending upon the source of receptor, radiolabelled ligand, and other components, as well as specific assay conditions. Thus, Compound A is included in all assays as a standardization control. That is, the values of binding affinities obtained for Compound A are compared to values reported below in Example 2, i.e. K_i = 3 X 10^{-10} M for 5-HT_{1A} receptors and 3 X 10^{-7} M for α_1-adrenergic receptors, and the values obtained in the same assay for other test compounds are normalized proportionately.

Measurement of pre-synaptic and post-synaptic serotoninergic 5-HT_{1A} receptor antagonist activity may be achieved using neurophysiological assay methods. For example, Raphe cell firing measured electrophysiologically is used as an index of pre-synaptic 5-HT_{1A} receptor activity (VanderMaelen et al., *Brain Res.*, 289, 109, 1983). In this assay, a 5-HT_{1A} receptor agonist acting at pre-synaptic somatodendritic 5-HT_{1A} receptors inhibits Raphe neuronal firing, which is detected by measuring the electrical activity of 5-HT-containing neurons. Antagonists prevent the inhibitory action of the 5-HT_{1A} receptor agonist, resulting in the maintenance of high levels of serotonin in the synaptic cleft. An alternative system for measuring pre-synaptic activity is the inhibition of hypothermia induced in mice by 8-OH-DPAT (Moser, *Eur. J. Pharmacol.*, 193, 165, 1991).

Inhibition of adenylate cyclase activity in rat hippocampal slices is used as an indicator of post-synaptic 5-HT_{1A} receptor activity (Shenker et al.,*Eur.J.Pharmacol.*, 109, 427, 1985). In this assay, compounds exhibiting antagonistic activity at post-synaptic 5-HT_{1A} receptors antagonize the inhibitory effects of a 5-HT_{1A} agonist only on forskolin-stimulated adenylate cyclase activity and display no intrinsic effect on the basal activity of the enzyme. Alternative methods for measuring post-synaptic activity include inhibition of 8-OH-DPAT induced behavioural syndrome, in particular the forepaw treading symptom (Tricklebank et al., *Eur. J. Pharmacol.*, 117, 15, 1985). These and other methods are reviewed in Fletcher et al., *TiPS*, 14, 441, 1993.

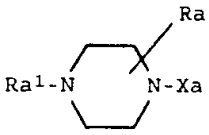
As discussed above, Compound A is included in all assays as a positive control; the values obtained for the pre-synaptic and post-synaptic antagonistic activity of Compound A are normalized proportionately with those disclosed below in Examples 6 and 7 (ID_{50} for pre-synaptic = 8.5 μg/Kg; post-synaptic = 14 μg/Kg), and the values obtained for other test compounds are compared.

Once a compound is identified as possessing 5-HT_{1A} receptor antagonist activity, its pharmacological activity is confirmed using one or more animal model systems for lower urinary tract disorders. Useful animal model systems include isovolumetric rhythmic bladder voiding contractions in anaesthetized rats and cystometry in conscious rats. In the first method, the urinary bladder is catheterized, ligated, and connected with a pressure recording device. The bladder is then filled until reflex voiding contractions occur, after which the frequency and amplitude of the voiding contractions are measured. In the second method, bladder volume capacity and micturition pressure are measured one day following bladder catheterization. In the first method, the test

compounds are administered intravenously prior to the measurements. Both oral and intravenous administration routes may be used in a second method. These methods are described in more detail in Examples 8 and 9 below, and were originally used to validate the predictive qualities of the true serotoninergic 5-HT_{1A} receptor antagonists for the foregoing urinary tract disorders.

5-HT_{1A} receptor antagonist compounds for use in the invention include compounds of the general formulae I to VII discussed below.

Piperazine derivatives of the general formula I



(I)

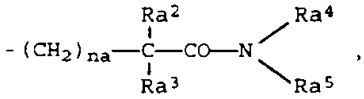
In these compounds:

Ra represents a hydrogen atom or a lower alkyl group;

Ra^1 represents an aryl, nitrogen-containing heteroaryl or nitrogen-containing bicyclic heteroaryl group; and

Xa represents one of the groups

(Aa)



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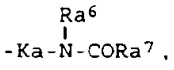
(Ba)



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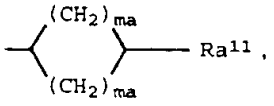
(Ca)



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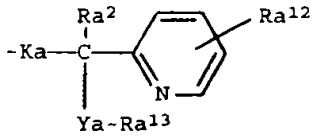
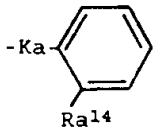
(Da)



and

(Ea)



;

na is 1 or 2;
ma is 1, 2 or 3;
Ra^2 represents a hydrogen atom or a lower alkyl group;
Ra^3 represents an aryl or aryl(lower)alkyl group;
Ra^4 represents a hydrogen atom or a C_1-C_3 alkyl group;
Ra^5 represents a hydrogen atom or a C_1-C_3 alkyl, C_3-C_{12} cycloalkyl or cycloalkyl(lower)alkyl group; or Ra^4 and Ra^5 together with the nitrogen atom to which they are attached represent a 1-azetidinyl, 1-pyrrolidinyl, piperidino, 1-perhydroazepinyl, morpholino, or 1-piperazinyl group, each optionally substituted by a lower alkyl, aryl or aryl(lower)alkyl group;
Ra^6 represents a monocyclic or bicyclic heteroaryl group;
Ra^7 represents a hydrogen atom, a lower alkyl, cycloalkyl, cycloalkenyl, cycloalkyl(lower)alkyl, aryl, aryl(lower)alkyl, heteroaryl or heteroaryl(lower)alkyl group, or a group $-NRa^8Ra^9$ or ORa^{10};
Ra^8 represents a hydrogen atom or a lower alkyl, aryl or aryl(lower)alkyl group;
Ra^9 represents a hydrogen atom or a lower alkyl, -CO-(lower)alkyl, aryl, -CO-aryl, aryl(lower)alkyl, cycloalkyl or cycloalkyl(lower)alkyl group; or Ra^8 and Ra^9 together with the nitrogen atom to which they are attached represent a saturated heterocyclic group which may contain an additional heteroatom;
Ra^{10} represents a lower alkyl, cycloalkyl, cycloalkyl -(lower)alkyl, aryl, aryl(lower)alkyl, heteroaryl or heteroaryl(lower)alkyl group;
Ra^{11} represents an aryl or nitrogen containing heteroaryl group;
Ra^{12} represents a hydrogen atom or a lower alkyl group;
Ra^{13} represents a hydrogen atom or a C_1-C_3 alkyl, C_3-C_{12} cycloalkyl or cycloalkyl(lower)alkyl group;
Ra^{14} represents an aryl group;
Ka represents a C_2-C_4 alkylene group optionally substituted by one or more lower alkyl groups; and
Ya represents a carbonyl, alkylene, hydroxyalkylene or hydroxycycloalkylene group or a group $-S(O)_{oa}$; where oa = 0 to 2.

When Ra^1 represents an aryl group, it preferably represents a phenyl group having a substituent in the ortho position or a 1-naphthyl group optionally substituted in the 2 or 7 positions. Examples of aryl groups Ra^1 are o-(lower)alkoxyphenyl, such as o-methoxyphenyl, or (lower)alkoxy substituted 1-naphthyl. When Ra^1 represents a bicyclic heteroaryl group, it preferably represents a 4-indolyl group.

When Ra^6 represents a bicyclic heteroaryl group, both rings can contain hetero ring atom(s), or only one ring can contain hetero atom(s). In the latter instance, the group Ra^6 is connected to the compound of formula (I) via the ring containing the hetero atom(s).

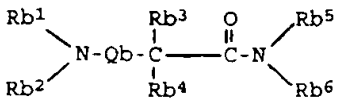
Examples of the heteroaryl group Ra^6 include monocyclic groups containing one hetero atom, such as pyridyl (particularly 2-pyridyl); monocyclic groups containing two hetero atoms, such as thiazolyl (particularly 2-thiazolyl); and bicyclic groups containing one or two hetero atoms, such as quinolinyl or isoquinolinyl and particularly 2-quinolinyl.

When Ra^{11} and Ra^{14} represent aryl groups, the preferred groups are phenyl. When Ra^{11} represents a heteroaryl group it is preferably a pyridyl group, optionally substituted by one or more alkyl groups.

Methods for the preparation of the piperazine derivatives I are disclosed in the following references: GB 2230780 (EP 395313), GB 2230781 (EP 395312), GB 2248836, (EP 481744), GB 2255337, GB 2262093, WO 94/15919, WO 94/15928, WO 94/21610, WO 95/33743 and GB 2277517.

Preferred piperazine derivatives I include:
1-{4-[4-(2-methoxyphenyl)-1-piperazinyl]-3-phenylbutanoyl}-perhydroazepine,
1-{4-[4-(2-methoxyphenyl)-1-piperazinyl]-2-phenylbutanoyl}-perhydroazepine,
1-[N-cyclohexylcarbonyl-N-(2-pyridyl)-2-aminoethyl)-4-(2-methoxyphenyl)-piperazine (Compound A),
1-[N-cyclohexylcarbonyl-N-(2-pyridyl)-2-aminoethyl)-4-(4-indolyl)-piperazine (Compound B),
1-[N-[2-(2-pyridylamino)-ethyl]-4-(2-methoxyphenyl)-piperazine (Compound C),
1-[N-[2-(2-pyridylamino)-ethyl]-4-(4-indolyl)-piperazine (Compound D), and
1-[2-(2-biphenyl)-ethyl]-4-(2-methoxyphenyl)-piperazine,
and their pharmaceutically acceptable acid addition salts.

Compounds of the general formula II



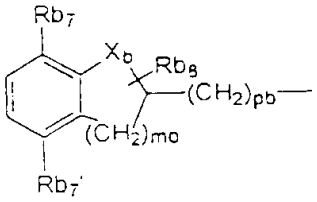
(II)

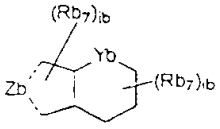
In these compounds:

Qb represents a C_1.C_3 alkylene group, optionally substituted by one or more lower alkyl groups;

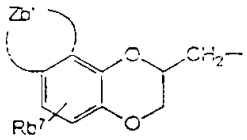
Rb^1 represents a hydrogen atom or a lower alkyl group;

Rb^2 represents one of the groups

(Ab) 

(Bb) 

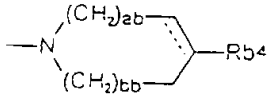
(Cb) Rb^9-CH_2CH_2- ,

(Db) 

(Eb) Rb^{10}-O-$CH_2CH(OH)CH_2$- and

(Fb) Rb^{10}-O-CH_2CH_2-;

or Rb^1 and Rb^2 together with the nitrogen atom to which they are attached represent a group of the formula



Rb^3 represents a hydrogen atom or a lower alkyl group;

Rb^4 represents an aryl, bicyclic aryl or heteroaryl group;

Rb^5 represents a hydrogen atom or a lower alkyl group;

Rb^6 represents a hydrogen atom or a C_1.C_{10} alkyl, C_3.C_{12} cycloalkyl, cycloalkyl(lower)alkyl, aryl or aryl(lower)alkyl group;

or Rb^5 and Rb^6 together with the nitrogen atom to which they are attached represent a saturated heterocyclic group, optionally containing an additional hetero atom and

optionally substituted by a halogen atom or a lower alkyl, aryl, aryl(lower)alkyl, lower alkoxy or halo(lower)alkyl group;

ab is 0 to 3 and bb is 0 to 3 but (ab + bb) is not more than 3;

---- represents an optional double bond which can be present provided that ab is at least 1;

Xb represents a group $-(CH_2)_{nb}-$, $-OCH_2-$ or $-SCH_2-$;

mb is 0 or 1, nb is 1 to 3, and pb is 0 or 1; provided that (mb + pb) is 1 and (mb + nb) is not more than 3;

Rb^7 represents a hydrogen or halogen atom, or a lower alkyl, (lower)alkylcarbonyl, lower alkoxy, (lower)alkoxycarbonyl, hydroxy, trifluoromethyl, carboxamido, nitro, cyano, amino, (lower)alkylamino or di(lower)alkylamino group;

$Rb^{7'}$ represents a hydrogen or halogen atom; with the proviso that when Xb represents a group $-OCH_2-$ or $-SCH_2-$ then $Rb^{7'}$ represents a hydrogen atom;

Rb^8 represents a hydrogen atom or a lower alkyl group;

ib = 0, 1 or 2; jb = 0, 1 or 2;

Yb represents an oxygen or sulphur atom or a methylene group;

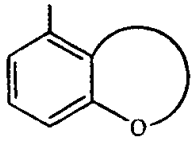
Zb represents the atoms necessary to form a heteroaromatic ring having from 5 to 7 carbon atoms fused to the non-aromatic ring containing the Yb group;

Rb^9 represents a monocyclic or bicyclic heteroaryl group;

Zb' represents either a pair of hydrogen atoms or the atoms necessary to form an aromatic or heteroaromatic ring fused to the benzodioxanyl group; and

Rb^{10} represents a monocyclic or bicyclic aryl or bicyclic heteroaryl group.

When Rb^4 represents a heteroaryl group, it is preferably a bicyclic oxygen-containing group of the formula:



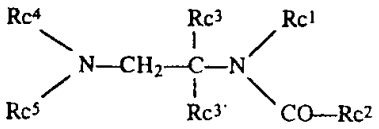
wherein the heterocyclic ring has from 5 to 7 ring atoms, is saturated or unsaturated, and optionally includes one or more hetero ring atoms or groups, such as -O-, -S-, $-SO_2-$ or NRb^3, in addition to the oxygen atom illustrated.

Examples of saturated heterocyclic groups which NRb^5Rb^6 may represent include 1-azetidinyl, 1-pyrrolidinyl, piperidino, 1-perhydroazepinyl, morpholino, 1-perhydroazocinyl and 1-piperazinyl groups.

The preparation of compounds II is disclosed in WO 94/03444 and WO 94/20481.

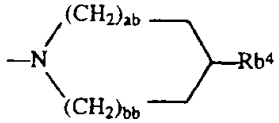
A preferred compound II is:
1-{4-[(1,4-benzodioxan-2-yl)-methylamino]-2-phenylbutanoyl}-perhydroazepine.

Compounds of the general formula III



(III)

In these compounds:
Rc^1 represents a heteroaryl or bicyclic heteroaryl group;
Rc^2 represents a cycloalkyl group;
Rc^3 represents a hydrogen atom or a lower alkyl group;
$Rc^{3'}$ represents a hydrogen atom or a lower alkyl group;
Rc^4 represents a hydrogen atom or a lower alkyl group; and
Rc^5 represents one of the groups (Ab), (Bb), (Cb), (Db), (Eb) and (Fb) as above defined;
or Rc^4 and Rc^5 together with the nitrogen atom to which they are attached represent a group of the formula

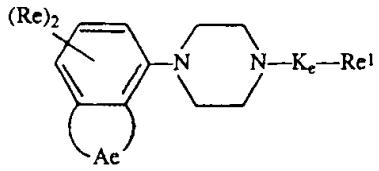


wherein ab, bb, Rb^4 and —— are as above defined.

The compounds III and their methods of preparation are disclosed in WO 94/21611 and WO 95/02592.

Preferred compounds having formula III are:
N-(1,4-benzodioxan-2-ylmethyl)-N-methyl-N'-(2-pyridyl)-N'-cyclohexylcarbonyl--1,2-diaminoethane,
(R)-4-(2-methoxyphenyl)-1-[N-cyclohexanoyl-N-(2-pyridyl)-3-amino-2-propyl]--piperidine, and
(R)-4-(2-thienyl)-1-[N-cyclohexanoyl-N-(2-pyridyl)-3-amino-2-propyl]--1,2,3,6-tetrahydropyridine.

Compounds of the general formula IV



(IV)

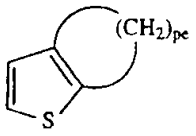
In these compounds:

Ae represents a group -OCH=CH-, $-OCH_2CH_2-$, $-OCH_2O-$, $-OCH_2CH_2O-$ or -OCOCH=CH-;

each Re independently represents a hydrogen or halogen atom or an alkyl, hydroxy, alkoxy, trifluoromethyl or cyano group;

Ke represents a C_1-C_8 linear or branched alkylene group, optionally substituted by an aryl or heteroaryl group;

Re^1 represents a phenyl, thienyl, naphthyl or benzothiophenyl group, or a group of the formula



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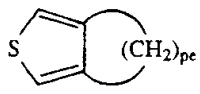

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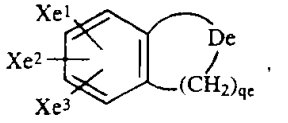

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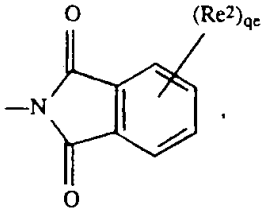

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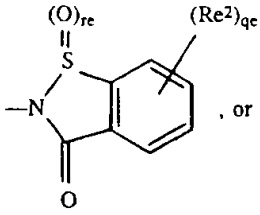

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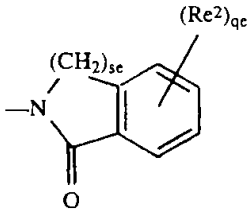
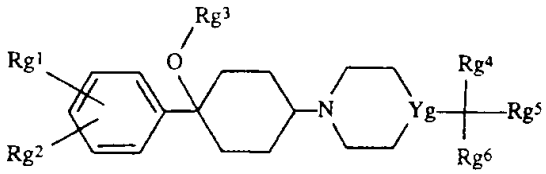


wherein pe is 3 or 4; qe is 0 to 3; re is 0 to 2; se is 1 or 2;
each Re^2 independently represents a halogen atom or an alkyl, hydroxy, alkoxy, trifluoromethyl or cyano group;
De represents a group -CH=CH- or $(CH_2)_{2-4}$; and
each of Xe^1, Xe^2 and Xe^3 independently represents a hydrogen atom or an alkyl, alkoxy, hydroxy, alkylthio, trifluoromethyl, nitro, amino or acetamido group, or two of Xe^1, Xe^2 and Xe^3 together represent a group $-OCH_2O-$ or $-OCH_2CH_2O-$.

The preparation of compounds having formula (IV) is disclosed in EP 490772, EP 574313 and EP 633260.

Preferred compounds having formula IV include:
1-[5-(1,4-benzodioxanyl)]-4-[3-(3-thienyl)-propyl]-piperazine,
1-[5-(1,4-benzodioxanyl)]-4-[2-(1-indanyl)-ethyl]-piperazine, and
1-[5-(1,4-benzodioxanyl)]-4-[3-(1-benzocyclobutyl)-propyl]-piperazine.

Compounds of the general formula V

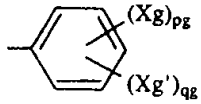


(V)

In these compounds:
each of Rg^1 and Rg^2 independently represents a hydrogen or halogen atom or a trifluoromethyl or C_1-C_4 alkoxy group;
or Rg^1 and Rg^2, being on adjacent carbon atoms, together represent a group of formula $-O(CH_2)_{ig}O-$ wherein ig is from 1 to 3;
each of Rg^3, Rg^4 and Rg^5 independently represents a hydrogen atom or a C_1-C_4 alkyl group or a phenyl group;
Yg represents a nitrogen atom or a group CH; and
Rg^6 represents a heteroaryl, phenyl or substituted phenyl group.

The preferred substituted phenyl groups which Rg^6 may represent have the formula Ag:

(Ag)



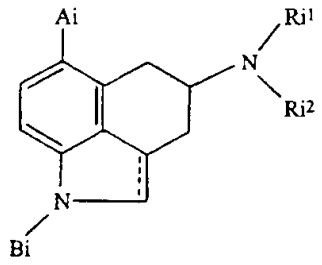
wherein pg is 0 to 5 and qg is 0 to 5 but (pg + qg) is not more than 5;
each of Xg and Xg' independently represents a halogen atom or a nitro, amino, carboxamido, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 haloalkyl, C_1-C_4 alkylthio or the like;
or Xg and Xg', being on adjacent carbon atoms, together represent a group $-O(CH_2)_{ng}O-$ wherein ng is 1 to 3.

The preferred heteroaryl groups which Rg^6 may represent include 3-pyridyl, 4-pyridyl, 2-thienyl, 2-furanyl and 1-methyl-2-pyrrolyl groups.

The preparation of compounds having formula V is disclosed in US 5387593 and EP 546583.

Preferred compounds having formula V include:
Z- and E-4-benzyl-1-[4-hydroxy-4-(1,4-benzodioxan-6-yl)-cyclohexyl]-piperazine, and
Z-4-(3-methoxybenzyl)-1-[4-methoxy-4-(1,3-benzodioxolan-5-yl)-cyclohexyl]-
-piperidine.

Compounds of the general formula VI



(VI)

In these compounds:
---- represents a single or a double bond;
Ri^1 represents a hydrogen atom, a C_1-C_4 alkyl, C_3-C_4 alkenyl, phenyl(C_1-C_4)alkyl or cyclopropylmethyl group, or a group $CORi^4$, $-(CH_2)_{ni}S(C_1$-$C_4)$alkyl or $-(CH_2)_{ni}C(O)NRi^9Ri^{10}$;
Ri^2 represents a hydrogen atom or a C_1-C_4 alkyl, C_3-C_4 alkenyl or cyclopropylmethyl group;
Ai represents a tetrazolyl or substituted tetrazolyl group, a heteroaryl group having 5 or 6 ring atoms of which from 1 to 3 may be oxygen, sulphur or nitrogen atoms, or a group $Qi{=}C(\mathrm{H})\text{—}Xi$,

Bi represents a hydrogen atom, a C_1-C_4 alkyl group or an amino-blocking group;
Xi represents a hydrogen atom or a group -ORi^3, -SRi^3 or -NRi^{5}Ri^6;
Ri^3 represents a C_1.C_3 alkyl, substituted C_1.C_3 alkyl, aryl, substituted aryl, aryl(C_1.C_4)alkyl, substituted aryl(C_1.C_4)alkyl or C_3.C_7 cycloalkyl group;
Ri^4 represents a hydrogen atom or a C_1-C_4 alkyl, C_1-C_4 haloalkyl, C_1-C_4 alkoxy or phenyl group;
each of Ri^5 and Ri^6 independently represents a hydrogen atom or a C_1-C_4 alkyl, phenyl(C_1-C_4)alkyl or phenyl group;
or Ri^5 and Ri^6 together with the nitrogen atom to which they are attached represent a C_3-C_5 heterocyclic ring;
each of Ri^9 and Ri^{10} independently represents a hydrogen atom or a C_1-C_4 alkyl or C_5-C_8 cycloalkyl group;
ni is 1 to 4; and
Qi represents an oxygen or sulphur atom.

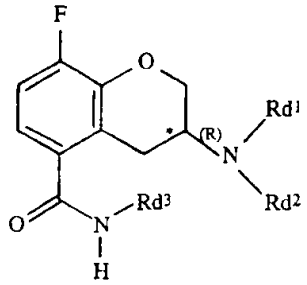
The term "amino-blocking group", as used herein, refers to a group which will prevent an amino group from participating in a reaction carried out on another functional group in the molecule, but which can be removed from the amine when desired. Such groups are described by T.W. Greene in chapter 7 of "*Protective Groups in Organic Synthesis*", John Wiley and Sons, New York, 1981. Groups which are useful for the compounds of the invention include benzyl and substituted benzyl groups such as 3,4-dimethoxybenzyl, o-nitrobenzyl and triphenylmethyl; groups having the formula COOR wherein R includes groups such as methyl, ethyl, propyl, isopropyl, 2,2,2-trichloroethyl, 1-methyl-1-phenylethyl, isobutyl, t-amyl, vinyl, allyl, phenyl, benzyl, p-nitrobenzyl, o-nitrobenzyl and 2,4-dichlorobenzyl; acyl and substituted acyl groups such as formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, trifluoroacetyl, benzoyl and p-methoxy-benzoyl; substituted sulphonyl groups such as methanesulphonyl, p-toluenesulphonyl, p-bromobenzene-sulphonyl, p-nitrophenylethyl and p-toluenesulphonyl-aminocarbonyl. Preferred amino-blocking groups are benzyl, acyl [C(O)R] or SiR_3 where R is C_1.C_4 alkyl, halomethyl or 2-halo-substituted-(C_2-C_4)alkoxy.

The preparation of compounds having formula VI is disclosed in EP 444854, EP 590971 and US 4576959.

Preferred compounds having formula VI include:
(2aS,4R)-4-(di-n-propylamino)-6-carbamoyl-1,2,2a,3,4,5-hexahydrobenz[c,d]indole,

(2aS,4R)-4-(di-n-propylamino)-6-dimethylcarbamoyl-
-1,2,2a,3,4,5-hexahydrobenz[c,d]indole,
(4R)-6-(5-isoxazolyl)-4-(di-n-propylamino)-1,3,4,5-tetrahydrobenz[c,d]indole,
(4R)-6-(2-oxazolyl)-4-(di-n-propylamino)-1,3,4,5-tetrahydrobenz[c,d]indole,
(4R)-6-(5-oxazolyl)-4-(di-n-propylamino)-1,3,4,5-tetrahydrobenz[c,d]indole, and
(4R)-6-[2-(1,3,4-oxadiazolyl)]-4-(di-n-propylamino)-1,3,4,5-tetrahydrobenz[c,d]indole.

Compounds of the general formula VII



(VII)

In these compounds:
Rd^1 represents an n-propyl or cyclobutyl group;
Rd^2 represents an isopropyl, t-butyl, cyclobutyl, cyclopentyl or cyclohexyl group; and
Rd^3 represents a hydrogen atom or a methyl group.

Compounds of the general formula VII may be prepared according to the methods described in WO 95/11891.

Preferred compounds of the general formula VII include:
(R)-5-carbamoyl-3-(N,N-dicyclobutylamino)-8-fluoro-3,4-dihydro-2H-1-benzopyran,
(R)-3-[N-(t-butyl)-N-(n-propyl)-amino]-5-carbamoyl-8-fluoro-3,4-dihydro-
-2H-1-benzopyran, and
(R)-5-carbamoyl-3-(N-cyclobutyl-N-isopropylamino)-8-fluoro-3,4-dihydro-
-2H-1-benzopyran.

As used herein, lower alkyl indicates alkyl groups having from 1 to 6 carbon atoms and preferably from 1 to 4 carbon atoms. Lower alkenyl as used herein indicates alkenyl groups having from 2 to 6 carbon atoms and preferably from 2 to 4 carbon atoms. The cycloalkyl groups contain from 3 to 12 carbon atoms and preferably from 5 to about 7 ring atoms. Cycloalkyl groups also include bicyclic, tricyclic and tetracyclic groups, such as adamantyl.

As used herein, aryl refers to aromatic groups having from 6 to 12 carbon atoms, such as phenyl and naphthyl. These may be substituted by one or more substituents. Preferred substituents include halogen atoms and lower alkyl, lower alkoxy, halo(lower)alkyl, nitro, cyano, amido, (lower)alkoxycarbonyl; amino; (lower)alkylamino; and di(lower)alkylamino groups. Two adjacent substituents on the aromatic ring can together to form a further fused ring, e.g. benzodioxanyl. The term halogen refers to fluorine, chlorine, and bromine. The preferred halogens are chlorine and fluorine. Examples of the preferred aryl(lower)alkyl groups include benzyl and phenethyl, optionally substituted as described above.

As used herein, heteroaryl refers to an aromatic group containing one or more hetero atoms (e.g. oxygen, nitrogen, or sulphur) and which can be monocyclic or bicyclic. The monocyclic heteroaryl group refers to an aromatic ring containing one or more nitrogen or other heteroatoms, such as pyridyl, 2-thienyl, 2-furanyl, 1-methyl-2-pyrrolyl, pyrimidinyl, pyrazinyl, oxazolyl, thiazinyl and the like. Preferred heteroaryl groups include 2-pyridyl, 3-pyridyl and 4-pyridyl groups. The heteroaryl groups may be substituted by halogen atoms or lower alkyl, lower alkoxy, halo(lower)alkyl, nitro, amino, cyano, amido, (lower)alkoxycarbonyl, (lower)alkylamino, and di-(lower)alkylamino groups. Bicyclic heteroaryl refers to phenyl rings fused with a second ring containing one or more heteroatoms. A particularly preferred heteroatom is nitrogen. Examples of the bicyclic heteroaryl groups include indazolyl, quinolinyl, isoquinolinyl and indolyl. The bicyclic heteroaryl groups can be substituted by one or more substituents. A preferred bicyclic heteroaryl group is indolyl substituted with alkoxycarbonyl groups.

The following Examples illustrate the invention. Reference is made in the Examples to the accompanying drawings, of which:

Figure 1 is a graphic illustration of a typical recorder tracing showing the effect of Compound A on volume-induced contractions of anaesthetized rats, the arrow indicating the intravenous administration of 300 μg/kg of Compound A, and

Figure 2 is a graphic illustration of a typical recorder tracing showing the effect of Compound A on cystometrographic parameters in conscious rats, the arrow indicating oral treatment of the animal with 3 mg/kg of Compound A.

Example 1

1-[N-(2-pyridyl)-2-aminoethyl]-4-(2-methoxyphenyl)-piperazine (Compound C)

23.5 g of 1-(2-aminoethyl)-4-(2-methoxyphenyl)-piperazine [Hexachemie-Reuil Malmaison-France] and 4.85 ml of 2-chloropyridine were stirred at 160°C in a closed reaction vessel for 10.5 hours. The reaction mixture was cooled to room temperature, dissolved in 320 ml of chloroform and washed with 1N sodium hydroxide (3 x 320 ml), followed by water (2 x 400 ml). The organic layer was dried on sodium sulphate and then evaporated to dryness under reduced pressure. The crude product was purified by column flash chromatography eluting with an ethyl acetate : 3N NH_3 in methanol 100:2 mixture affording, after evaporation of the collected fractions, 5 g of the title compound as an oil. A sample was crystallized from ethyl acetate to give a solid melting at 89-94°C.

^{1}H-NMR ($CDCl_3$, δ)

8.08, ddd, 1H, CH at position 6 in the pyridine ring
7.40, ddd, 1H, CH at position 4 in the pyridine ring
6.80-7.05, m, 4H, 2-methoxyphenyl CHs
6.55, ddd, 1H, CH at position 5 in the pyridine ring
6.40, dd, 1H, CH at position 3 in the pyridine ring
5.10, bs, 1H, NH
3.85, s, 3H, CH_3O
3.38, dt, 2H, CH_2NH
3.00-3.15, m, 4H, piperazine CHs
2.60-2.75, m, 6H, piperazine CHs and CH_2N
D_2O addition makes NH signal appear upfield as HDO.

Example 2

N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl}-N-(2-pyridyl)-
-cyclohexanecarboxamide . 2.66 HCl . 0.33 H_2O (Compound A)

To a solution of 4.26 g of the compound prepared in Example 1 in 42.5 ml of tetrahydrofuran, 6.52 ml of 2.5N n-butyllithium (hexane solution) was added dropwise at -22°C. After 40 minutes stirring at -20°C, 2.21 ml of cyclohexanecarbonyl chloride was added dropwise. The reaction mixture was stirred at -20°C for 20 minutes, then at room temperature for 3.5 hours. Water was cautiously added to quench the reaction, followed by 3N sodium hydroxide. Ethyl acetate extraction followed by washing the organic layer with water, drying on sodium sulphate and evaporating the solvent to dryness under reduced pressure gave an oily crude which was purified by column flash chromatography eluting with an ethyl acetate : 3N NH_3 in methanol 100:2 mixture. Evaporation of the collected fractions afforded 5 g of the title compound as a base, which was converted into the hydrochloride by dissolution in methanol and addition of excess 2.8N HCl in diethyl ether. Evaporation to dryness of the solvents and desiccation of the solid in vacuo yielded 5.30 g of the title compound. M.p. 161-164°C.

Elemental analysis for $C_{25}H_{34}N_4O_2$. 2.66 HCl . 0.33 H_2O:
calc. (%): C 57.14, H 7.16, N 10.66, Cl 17.95, H_2O 1.13
found (%): C 57.45, H 7.29, N 10.67, Cl 18.13, H_2O 1.20

^{1}H-NMR (D_6-DMSO, δ):

11.10-11.70, bs, 1H, NH+
8.58, dd, 1H, CH at position 6 in the pyridine ring
8.05, ddd, 1H, CH at position 4 in the pyridine ring
7.64, dd, 1H, CH at position 3 in the pyridine ring
7.45, dd, 1H, CH at position 5 in the pyridine ring
6.82-7.10, m, 4H, 2-methoxyphenyl CHs
5.20-5.80, bs, 2.4H, NH+ (remaining), H_2O
4.17, t, 2H, CH2NCO
3.79, s, 3H, CH_3O
3.00-3.75, m, 10H, CH_2N and piperazine CHs
2.15-2.35, m, 1H, cyclohexane CHCO
0.85-1.85, m, 10H, cyclohexane CHs
D_2O addition makes NH signals appear upfield as HDO.

Example 3
N-{2-[4-(4-Indolyl)-1-piperazinyl]-ethyl}-N-(2-pyridyl)-cyclohexanecarboxamide (Compound B)

Step a:
N-(2,2-Dimethoxyethyl)-N-(2-pyridyl)-cyclohexanecarboxamide
13.2 ml of a 2.5M solution of butyl lithium in n-hexane was added to a solution of 6g of 2-(2-pyridylamino)-acetaldehyde dimethyl acetal (prepared as described in Beilstein E III/IV, *22*, 3871) in 40 ml of tetrahydrofuran stirred at 0°C and the resulting mixture was stirred at room temperature for 1 hour. 4.46 ml of cyclohexanecarbonyl chloride was then added dropwise over a period of 5 minutes. Stirring was continued for 5.5 hours and the reaction mixture was then evaporated to dryness *in vacuo*. The residue was purified by flash chromatography, eluting with chloroform:ethyl acetate 7:3, to afford 13.3 g of the title compound.

^{1}H-NMR ($CDCl_3$, δ):

8.48-8.54, m, 1H, CH at position 6 in the pyridine ring
7.75, ddd, 1H, CH at position 4 in the pyridine ring
7.18-7.44, m, 2H, CHs at positions 3 and 5 in the pyridine ring
4.65, t, 1H, $C\underline{H}CH_2$
3.90, d, 2H, CH_2
3.31, s, 6H, 2 x CH_3O
2.32, tt, 1H, $C\underline{H}(CH_2)_2$
0.80-1.85, m, 10H, cyclohexane CH_2s

Step b:
N-Formylmethyl-N-(2-pyridyl)-cyclohexanecarboxamide
A mixture of 1.46 g of N-(2,2-dimethoxyethyl)-N-(2-pyridyl)--cyclohexanecarboxamide, 0.05 g of 1,4-hydroquinone and 25ml of 2N HCl was stirred at 80°C for 20 minutes under a nitrogen stream. The mixture was then cooled to 0°C, diluted with 50ml of dichloromethane, and adjusted to pH 10 by addition of a 20% solution of sodium carbonate. The aqueous layer was extracted twice with dichloromethane and the combined organic layers were dried over anhydrous sodium sulphate. Evaporation to dryness *in vacuo* gave 0.94 g of the title compound, used without purification in the next reaction step.

^{1}H-NMR ($CDCl_3$, δ):

9.66, s, 1H, CHO
8.48-8.54, m, 1H, CH at position 6 in the pyridine ring
7.79, ddd, 1H, CH at position 4 in the pyridine ring
7.18-7.44, m, 2H, CHs at positions 3 and 5 in the pyridine ring
4.52, s, 2H, CH_2
2.48, tt, 1H, $C\underline{H}(CH_2)_2$
0.80-1.95, m, 10H, cyclohexane CH_2s

Step c:

N-{2-[4-(4-Indolyl)-1-piperazinyl]-ethyl}-N-(2-pyridyl)-cyclohexanecarboxamide HCl . $1.25H_2O$

A mixture of 0.94 g of N-formylmethyl-N-(2-pyridyl)-cyclohexanecarboxamide, 0.69 g of 1-(4-indolyl)-piperazine, 1.21 g of sodium triacetoxyborohydride, 0.44 ml of acetic acid and 30 ml of 1,2-dichloroethane was stirred at room temperature for 3 hours. The mixture was then diluted with 20 ml of water and adjusted to pH 10 by addition of a 20% solution of sodium carbonate. The aqueous layer was extracted twice with 1,2-dichloroethane and the combined organic layers were dried over anhydrous sodium sulphate. Evaporation to dryness *in vacuo* gave a crude product which was purified by flash chromatography, eluting with dichloromethane:methanol 98:2 to 95:5, to give 0.96 g of the base of the title compound. This was dissolved in 40 ml of dichloromethane and 3.8N hydrogen chloride in diethyl ether was added. The title compound precipitated and was filtered off. Yield 0.66 g. M.p. 181-187°C.

Example 4

1-(4-Indolyl)-4-[2-(2-pyridylamino)-ethyl]-piperazine . 3HCl . $2H_2O$ (Compound D)

Step a:

2-[4-(4-Indolyl)-1-piperazinyl]-N-(2-pyridyl)-acetamide

A mixture of 1.4 g of 1-(4-indolyl)-piperazine, 1.26 g of 2-chloro-N-(2-pyridyl)--acetamide (prepared as described in Beilstein E III/IV, *22*, 3881), 1.3 ml of diisopropylethylamine and 14 ml of dimethylformamide was stirred at 60°C under a nitrogen stream for 4 hours. The mixture was then diluted with 200 ml of water and extracted with ethyl acetate (4 x 50 ml). The combined organic layers were washed with water, dried on anhydrous sodium sulphate and evaporated to dryness *in vacuo* to give 2.37 g of the title compound as a crude base. Crystallization from methanol afforded 1.6 g melting at 198-201°C.

Step b:

1-(4-Indolyl)-4-[2-(2-pyridylamino)-ethyl]-piperazine . 3HCl . $2H_2O$

0.34 g of 95% lithium aluminium hydride was added to a solution of 2-[4-(4-indolyl)-1-piperazinyl]-N-(2-pyridyl)-acetamide in 30 ml of anhydrous tetrahydrofuran stirred at room temperature. The resulting mixture was stirred under reflux for 10 hours, and subsequently cooled. It was then diluted with 7ml of 2N sodium hydroxide and 50ml of water and dried on anhydrous sodium sulphate. The solvents were evaporated off *in vacuo*, giving 0.94 g of an oily residue. Purification by flash chromatography, eluting with ethyl acetate:methanol 96:4 to 70:30, gave 0.76 g of the base of the title compound. This was dissolved in 20 ml of dichloromethane and an excess of 3.8N hydrogen chloride in diethyl ether was added. The title compound precipitated. It was filtered off and dried at 60°C (0.5 mm Hg). M.p. (127) 144-152°C.

Example 5

Measurement of Binding of Test Compounds to 5-HT_{1A} and α_1-Adrenergic Receptors

[3H] prazosin binding (α_1-receptors):

Rat cerebral cortices were homogenized in 50 volumes of ice-cold 50 mM Tris-HCl pH 7.4. The homogenates were centrifuged at 48,000 x g for 10 minutes, and the pellets were resuspended in the same volume of ice-cold buffer, centrifuged, and resuspended two more times. The final pellets were resuspended in 100 volumes of 50 mM Tris-HCl, pH 7.4, containing 0.1% ascorbic acid and 10 μM pargyline. 1-ml samples were incubated for 30 min at 25°C with 0.35 nM [^{3}H]prazosin, in the absence or presence of different concentrations (10^{-5} to 10^{-10} M) of the test compound. Non-specific binding was determined in the presence of 10 μM phentolamine. The incubations were terminated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester, after which the filters were washed with 3x3 ml of ice-cold buffer. The radioactivity retained on the filters was determined by liquid scintillation counting. The results are shown in Table 1 below.

[3H]8-OH-DPAT binding ($5HT_{1A}$ receptors):

Rat hippocampi were homogenized in 50 volumes of ice-cold 50 mM Tris-HCl pH 7.4. The homogenates were centrifuged at 48,000 x g for 10 minutes, and the pellets were resuspended in the same volume of ice-cold buffer, incubated for 10 minutes at 37°C, centrifuged and resuspended two more times. The final pellets obtained were resuspended in 100 volumes of 50 mM Tris-HCl, pH 7.4, containing 0.1% ascorbic

acid and 10 μM pargyline. 1 ml samples were incubated for 30 min at 25°C with 1 nM [^{3}H]8-OH-DPAT, in absence or presence of different concentrations (10^{-5} to 10^{-10} M) of the test compound. Non-specific binding was determined in the presence of 10 μM 5-HT. The incubations were terminated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester, after which the filters were washed with 3 x 3 ml of ice-cold buffer. The radioactivity retained on the filters was determined by liquid scintillation counting. The results are shown in Table 1 below.

TABLE 1

Binding affinity for the 5-HT_{1A} receptor and α_1-adrenergic receptor.

Data are expressed as Ki (nM).

Compound	5-HT_{1A} receptor	α_1-adrenergic receptor
Compound A	0.3	295.5
Compound B	0.13	458.3
Compound C	20.2	214.7
Compound D	16.3	89.2
NAN-190	1.9	4.8

These results indicate that Compound A and Compound B bind tightly and selectively to the 5-HT_{1A} receptor. By contrast, NAN-190 exhibits nearly equivalent binding to both receptors.

Example 6

Measurement of Pre-Synaptic 5-HT_{1A} Receptor Antagonist Activity

Antagonism of hypothermia induced by 8-OH-DPAT in mice:

The antagonistic effect of 5-HT_{1A} receptor antagonists on hypothermia induced by 8-OH-DPAT was evaluated by the method of Moser (Moser, *Eur. J. Pharmacol.*, 193, 165, 1991) with minor modifications.

Male CD-1 mice (28-38 g) obtained from Charles River (Italy) were housed in a climate-controlled room (temperature 22 $\pm$ 2°C; humidity 55 $\pm$ 15%) and maintained on a 12 h light/dark cycle with free access to food and water. On the day of experiment, mice were placed singly in clear plastic boxes under the same ambient

conditions. Body temperature was measured by the insertion of a temperature probe (Termist TM-S, LSI) into the rectum to a depth of 2 cm. Rectal temperature was measured immediately prior to subcutaneous injection of the test compound and 30 min later. All animals then received 8-OH-DPAT (0.5 mg/kg s.c.) and their temperature was measured 30 min later. For each animal, temperature changes were calculated with respect to pretreatment values and the mean values were calculated for each treatment group.

A linear regression equation was used in order to evaluate ID_{50} values, defined as the dose of antagonist needed to block 50% of the hypothermic effect induced by 0.5 mg/kg 8-OH-DPAT administered subcutaneously.

The results are shown in Table 2 below.

TABLE 2

Antagonistic activity for the pre-synaptic 5-HT_{1A} receptor.

COMPOUND	ID_{50} (95%) C.L. in μg/kg s.c.
Compound A	8.5 (5.8-12.5)
Compound B	1.9 (1.0-3.7)
NAN-190	not active

These results demonstrate that Compound A and Compound B have significant pre-synaptic 5-HT_{1A} receptor antagonist activity.

Example 7

Measurement of Post-Synaptic 5-HT_{1A} Receptor Antagonist Activity

Inhibition of forepaw treading induced by 8-OH-DPAT in rats:

The inhibitory effect of 5-HT_{1A} receptor antagonists on the forepaw treading induced in rats by subcutaneous injection of 8-OH-DPAT was evaluated by the method of Tricklebank (Tricklebank et al., *Eur.J. Pharmacol.*, 117: 15, 1985) with minor modifications.

Male Sprague-Dawley rats (150-175 g) obtained from Charles River (Italy), were housed in a climate-controlled room and maintained on a 12 h light/dark cycle with free access to food and water. On the day of experiment, rats were placed singly in clear plastic boxes. Reserpinised rats were treated with reserpine, 1 mg/kg s.c., 18-24

h before the test. For evaluation of antagonistic activity, compounds were i.p. or s.c. administered 16 min before 8-OH-DPAT (1 mg/kg s.c.). Observation sessions of 30 s duration began 3 min after treatment with the agonist and were repeated every 3 min over a period of 15 min.

The appearance of the forepaw treading symptom induced by post-synaptic stimulation of the $5HT_{1A}$ receptors was noted, and its intensity was scored using a ranked intensity scale in which: 0 = absent, 1 = equivocal, 2 = present and 3 = intense. Behavioural scores for each treated rat were accumulated over the time course (5 observation periods) and expressed as mean values of 8-10 rats.

A linear regression equation was used in order to evaluate ID_{50} values, defined as the dose of antagonist needed to block 50% of the forepaw treading intensity induced by 1 mg/kg 8-OH-DPAT administered subcutaneously.

The results are shown in Table 3 below.

TABLE 3

Compound	NORMAL RATS ID_{50} μg/Kg	RESERPINIZED RATS ID_{50} μg/Kg
Compound A (s.c.)	14 (12-16)	8.5 (5.8-12.5)
NAN-190 (i.p.)	700 (500-1000)	2000 (1600-2400)

These results demonstrate that Compound A exhibits significant post-synaptic $5\text{-}HT_{1A}$ receptor antagonist activity. NAN-190, by contrast, is much less active. Taken together, the bioassays for pre-synaptic and post-synaptic antagonist activity are effective for identifying compounds that exhibit both activities at levels that render them useful in treating urinary tract disorders.

Example 8

Effect of $5\text{-}HT_{1A}$ Receptor Antagonists on Volume-Induced Rhythmic Bladder Voiding Contractions in Anaesthetized Rats

Female Sprague Dawley rats weighing 225-275 g (Crl: CD° BR, Charles River Italia) were used. The animals were housed with free access to food and water and were maintained on a forced 12 h alternating light-dark cycle at 22-24°C for at least one week, except during the experiment. The activity on the rhythmic bladder voiding

contractions was evaluated according to the method of Dray (*J. Pharmcol. Methods*, 13:157, 1985), with some modifications as in Guarneri (*Pharmacol. Res.*, 27:173, 1993). Briefly, rats were anaesthetized by subcutaneous injection of 1.25 g/kg (5 ml/kg) urethane, after which the urinary bladder was cathetized via the urethra using PE 50 polyethylene tubing filled with physiological saline. The catheter was tied in place with a ligature around the external urethral orifice and was connected with conventional pressure transducers (Statham P23 ID/P23 XL). The intravesical pressure was displayed continuously on a chart recorder (Battaglia Rangoni KV 135 with DC1/TI amplifier). The bladder was then filled via the recording catheter by incremental volumes of warm (37°C) saline until reflex bladder voiding contractions occurred (usually 0.8-1.5 ml). Two parameters were recorded from the cystometrogram: the frequency of voiding contractions, calculated by counting the number of peaks/15 min of observation, and the mean amplitude of these contractions (mean height of the peaks in mmHg) in the same time period. For intravenous (i.v.) injection of bioactive compounds, a PE 50 polyethylene tubing filled with physiological saline was inserted into the jugular vein.

Bioactivity was assessed in individual animals (using 6-10 rats per dose), by recording the number and height of the peaks for 15 min after drug injection and comparing them with those previously recorded for 15 min after the intravenous administration of vehicle alone. In the evaluation of the mean amplitude of peaks after treatment, only the results from the cystometrograms showing a reduction in the frequency of contractions of $\leq 50\%$ were utilized. The statistical significance of changes in frequency and amplitude before and after treatment was assessed by Student's *t* test for paired data. Changes showing a probability $P < 0.01$ were considered significant.

An all-or-none criterion was also used to compare bioactivity in terms of ED_{50} values. Rats showing a treatment-related change of $\geq 30\%$ relative to the basal value were considered to be positive. Quantal dose-response curves and ED_{50} values were evaluated by the method of Bliss (*J. Pharm. Pharmacol.*, 11:192, 1938). In addition, since most compounds produced an effect that was relatively rapid in onset and led to a complete cessation of bladder contractions (as shown in Figure 1), bioactivity was conveniently estimated by measuring the duration of bladder quiescence (i.e., the duration of time during which no contractions occurred). To compare the potency of the tested compounds in inhibiting the frequency of the bladder voiding contractions, equieffective doses producing 10 minutes of disappearance time (ED_{10min}) were computed by means of least square linear regression analysis.

The rapid distension of the urinary bladder in urethane-anaesthetized rats produced a series of rhythmic bladder voiding contractions whose characteristics have been described (Maggie et al., *Brain Res.*, 380, 83, 1986; Maggi, et al., *J. Pharmacol. Exp. Ther.*, 230, 500, 1984). The frequency of these contractions is related to the sensory afferent arm of reflex micturition and to the integrity of the micturition centre, while their amplitude is a property of the efferent arm of the reflex (Maggi et al., *J. Pharmacol. Meth.*, 15, 157, 1986; Maggi et al., *Brain Res.*, 415, 1, 1987; Maggi et al., *Naun. Schmied. Arch. Pharmacol.*, 332, 276, 1986; Maggi et al., *J. Urol.*, 136, 696, 1986). In this model system, compounds that act mainly on the CNS (such as morphine) cause a reduction in the voiding frequency, whereas drugs that act at the level of the detrusor muscle lower the amplitude of the bladder contractions.

The results are tabulated in Tables 4 and 5 below.

TABLE 4

Effects on rhythmic bladder voiding contractions after intravenous administration.

Data represent the mean values ± S.E. of the number of contractions observed before and after the i.v. administration of the tested compounds, as well as the amplitude of the peaks recorded in animals showing a reduction of the frequency <50%. The ED50 (and 95% confidence limits) values were evaluated on the basis of a quantal criterion as described in the Methods.

COMPOUND Dose μg/kg i.v.	No. of rats	FREQUENCY No. contr./15 min before treatment	FREQUENCY No. contr./15 min after treatment	AMPLITUDE mm Hg before treatment	AMPLITUDE mm Hg after treatment
Compound A					
1	10	11.7±1.0	11.6±1.3	25.4±2.0	23.1±1.9*
3	10	11.5±0.7	9.1±1.0*	25.1±2.2	21.7±2.0*
10	10	11.5±1.5	5.9±1.6*	26.0±3.8	22.3±3.8
30	10	11.8±0.7	3.8±0.7*	28.5±2.5	25.0±4.0
100	10	12.0±0.9	4.1±1.1*	28.0±4.6	25.7±2.9
300	10	9.5±0.6	2.2±0.5*	n.c.	n.c.
ED_{50}(μg/kg)		5 (3 ÷ 8)		n.a.	
Compound B					
0.3	6	10.7±1.4	11.3±1.7	31.0±3.7	27.7±4.1
1	6	11.7±1.5	8.7±1.3	26.5±4.3	22.7±5.1*
3	6	9.7±0.8	4.7±1.1*	33.7±2.4	25.3±1.2
10	6	11.7±1.7	4.2±0.9*	19.0	17.0
30	6	12.0±1.1	4.5±1.4	n.c.	n.c.
ED_{50}(μg/kg)		1 (0.6 ÷ 2)		n.a.	

TABLE 4 continued

COMPOUND Dose µg/kg i.v.	No. of rats	FREQUENCY No. contr./15 min before treatment	FREQUENCY No. contr./15 min after treatment	AMPLITUDE mm Hg before treatment	AMPLITUDE mm Hg after treatment
Compound C					
30	6	9.7±1.0	7.2±1.3	29.6±7.4	24.0±6.2
100	6	9.7±1.0	4.5±1.6*	19.0±0.0	15.5±0.5
300	6	12.7±1.3	2.2±0.9*	n.c.	n.c.
1000	6	11.2±1.0	4.0±1.4*	40.0±0.0	25.5±0.5
ED_{50}(µg/kg)		54 (33 ÷ 91)		n.a.	
Compound D					
30	6	9.3±0.8	9.5±1.3	27.0±2.4	23.8±2.6*
100	6	15.3±1.0	9.3±2.8	27.0±1.5	23.7±1.5*
300	6	13.2±2.7	4.2±2.2*	26.0	20.0
1000	6	10.2±0.6	4.2±1.5*	32.0±5.0	23.5±5.5
ED_{50}(µg/kg)		77 (49 ÷ 122)		n.a.	
Flavoxate					
300	5	9.2±1.2	8.8±1.7	25.9±1.3	24.1±1.8
1000	17	10.1±0.7	8.6±0.9	26.1±2.3	25.6±2.2
3000	21	10.7±0.7	6.5±0.7*	18.9±0.9	16.6±1.2
10000	20	11.3±0.8	5.5±0.6*	19.9±1.6	19.2±1.5
ED_{50}(µg/kg)		2650 (1430 ÷ 4910)		n.a.	
Oxybutynin					
30	6	14.8±1.9	15.5±2.3	26.7±2.7	23.3±2.5
100	6	13.3±1.1	14.7±1.0	25.0±3.7	18.7±2.7*
300	12	10.1±0.7	8.8±0.8	20.9±1.7	13.6±0.8*
1000	13	9.7±0.8	9.5±0.8	20.3±2.4	11.8±1.2*
3000	13	10.0±0.7	9.9±1.5	18.0±1.4	10.8±1.0
ED_{50}(µg/kg)		n.a.		240 (140 ÷ 400)	
NAN-190(A)					
30	10	13.5±1.2	11.8±1.5	30.2±3.7	26.1±3.1*
100	10	13.6±1.0	6.4±1.1*	22.5±1.7	17.3±2.9
300	11	11.8±1.1	6.6±1.1*	24.1±1.8	17.6±1.7*
ED_{50}(µg/kg)		46 (23 ÷ 92)		n.a.	

* = $p \leq 0.01$ (Student's *t* test for paired data)
n.c. = not calculated
n.a. = not active on the parameter
A) = higher doses were not tested because of the high toxicity and low solubility of this compound.

TABLE 5

Effects on rhythmic bladder voiding contractions after intravenous administration.

Data represent the mean values ± S.E. of the duration of bladder quiescence (disappearance time of contractions in min). The ED_{10min} values represent the extrapolated dose inducing 10 min of disappearance of the contractions.

COMPOUND Dose µg/kg i.v.	No. of rats	BLADDER CONTRACTIONS Disappearance time (min)
Compound A		
1	10	1.34 ± 0.23
3	10	2.15 ± 0.42
10	10	8.13 ± 1.90
30	10	8.87 ± 1.08
100	10	12.56 ± 2.07
300	10	13.37 ± 1.83
ED_{10min}(µg/kg)		37 (18 ÷ 77)
Compound B		
0.3	6	1.10 ± 0.16
1	6	4.33 ± 1.30
3	6	7.58 ± 2.15
10	6	10.00 ± 0.92
30	6	8.85 ± 1.53
ED_{10min}(µg/kg)		9 (3 ÷ 24)
Compound C		
30	6	4.00 ± 1.87
100	6	9.60 ± 2.37
300	6	12.37 ± 2.63
1000	6	14.00 ± 4.45
ED_{10min}(µg/kg)		173 (28 ÷ 1087)
Compound D		
30	6	1.63 ± 0.50
100	6	6.55 ± 2.24
300	6	12.75 ± 2.45
1000	6	9.37 ± 2.44
ED_{10min}(µg/kg)		181 (89 ÷ 366)

TABLE 5 continued

COMPOUND Dose μg/kg i.v.	No. of rats	BLADDER CONTRACTIONS Disappearance time (min)
Flavoxate		
300	5	1.70 ± 0.60
1000	17	3.04 ± 0.96
3000	21	5.30 ± 1.00
10000	20	8.25 ± 1.90
ED_{10min}(μg/kg)		> 10000
NAN-190(A)		
30	10	1.80 ± 0.52
100	10	6.34 ± 1.18
300	11	5.47 ± 1.93
ED_{10min}(μg/kg)		> > 300

A) = higher doses were not tested because of the high toxicity and low solubility of this compound.

Compound B, after intravenous administration, dose dependently inhibited the frequency of the rhythmic bladder voidings and also reduced amplitude to some extent. Compound A, after intravenous administration, dose dependently inhibited the frequency of the rhythmic bladder voidings with no effect on their amplitude. The maximal change of this parameter, in fact, was about 13% and no dose-dependence was observed. Of the test compounds shown, Compound B was the most potent, being 46- and 2650-fold more active than NAN-190 and flavoxate, respectively. Compound A was 9- and 530-fold more active than NAN-190 and flavoxate, respectively.

By contrast, oxybutynin was only effective at reducing the amplitude of the contractions, confirming that its effects are due to a complete inhibition of the muscarinic receptors in the bladder.

The compounds that reduced the contraction frequency induced a complete and transient disappearance of contractions for a time period that was directly proportional to the administered dose (Table 5).

Tables 4 and 5 also illustrate the effects on volume-induced bladder contractions of flavoxate, a drug widely utilized in clinical therapy for bladder dysfunctions. Administration of this drug resulted in suppression of bladder contractions. The mean disappearance time observed after administration of the highest tested dose (10,000 μg/kg i.v.) was 8.25 ± 1.90 min. NAN-190 at the highest tested doses of 100-300 μg/kg gave a maximum disappearance time ranging from 5.5 to 6.3 min. (higher doses were not tested because of the high toxicity and low solubility of this compound).

These results indicate that Compound B and Compound A are potent compounds in reducing the frequency of the voiding contractions when compared to flavoxate and NAN-190 in terms of both absolute potency (ED_{50}) and disappearance time (ED_{10min}). Their mechanism of action appears to be different from that of oxybutynin, a peripheral antimuscarinic. Furthermore, their effects appeared at very low doses.

Example 9

Effect of 5-HT_{1A} Receptor Antagonists on Cystometric Parameters in the Conscious Rat

A. Methods:

Male Sprague Dawley rats (Crl: CD° BR) weighing 250-350 g were used. The animals were housed with free access to food and water and maintained on a forced 12 h alternating light-dark cycle at 22-24°C for at least one week, except during performance of the experiment.

To quantify urodynamic parameters in conscious rats, cystometrographic studies were performed using procedures described in Pietra et al., *IRCS Med. Sci.*, 14, 992, 1986; and Guarneri et al., *Pharmacol. Res.*, 24, 175, 1991.

Male rats were anaesthetized with nembutal (30 mg/kg) and chloral hydrate (125 mg/kg) i.p. and were placed in a supine position. An approximately 10 mm long midline incision was made in the shaved and cleaned abdominal wall. The urinary bladder was gently freed from adhering tissues, emptied, and then cannulated, via an incision at the dome, with a polyethylene cannula (Portex PP30), which was permanently sutured with silk thread. The cannula was exteriorized through a subcutaneous tunnel in the retroscapular area, where it was connected with a plastic adapter to avoid the risk of removal by the animal. For intravenous (i.v.) injection of test compounds, a PE 50 polyethylene tubing filled with physiological saline was inserted into the jugular vein and exteriorized in the retroscapular area.

Since cystometrographic parameters have been reported to be influenced by the time elapsed after catheter implantation Yaksh et al. *Amer. J. Physiol.*, 251, R1177, 1986 the rats were utilized exclusively one day after implantation.

On the day of the experiment, the rats were placed in Bollman's cages; after a stabilization period of 20 min, the free tip of the bladder catheter was connected through a T-shaped tube to a pressure transducer (Bentley T 800/Marb P 82) and to a peristaltic pump (Gilson minipuls 2) for a continuous infusion, at the constant rate of 0.1 ml/min, of saline solution into the urinary bladder. The intraluminal pressure signal during infusion was continuously recorded on a polygraph (Battaglia Rangoni KO 380 with ADC1/T amplifier). Two urodynamic parameters were evaluated: bladder volume capacity (BVC) and micturition pressure (MP). BVC (in ml) is defined as the minimum volume infused after which detrusor contraction (followed by micturition) occurs. MP (in mmHg) is defined as the maximal intravesical pressure induced by the contraction of detrusor during micturition. Basal BVC and MP values were calculated as the means of the first two recorded cystometrograms. At this point, the infusion was interrupted and the test compounds were administered. Fifteen minutes after intravenous administration, or one hour after oral drug administration, two additional cystometrograms were recorded in each animal and the mean values of the two cystometrographic parameters were calculated. A typical tracing is shown in Figure 2, where the effects of 3 mg/kg p.o. of Compound A are shown.

The statistical significance of the differences in urodynamic parameter values was evaluated by Student's *t* test for paired data. Only changes showing a probability $P < 0.01$ were considered to be significant.

B. Results:

The effects on urodynamic parameters in conscious rats after i.v. administration of different doses of Compound A and the reference compounds are summarized in Tables 6 and 7.

TABLE 6

Effects on cystometrogram in conscious rats.

Data represent the mean ± S.E. values (ml) of bladder volume capacity (BVC), before and 15 min after i.v. injection of the compounds.

COMPOUND Dose µg/kg i.v.	No. of rats	BVC before treatment	BVC after treatment	% change
CONTROL vehicle	12	0.50±0.09	0.43±0.06	-17
Compound A				
100	8	0.65±0.06	0.66±0.08	+4
300	9	0.47±0.05	0.63±0.06*	+32
1000	20	0.64±0.05	0.83±0.07*	+29
2000	10	0.48±0.04	0.63±0.05*	+32
Flavoxate				
300	17	0.76±0.11	0.87±0.11	+14
1000	14	0.88±0.15	1.11±0.15*	+26
3000	18	0.77±0.08	1.07±0.12*	+39
Oxybutynin				
100	13	0.82±0.15	0.89±0.18	+9
300	12	0.83±0.13	0.83±0.12	0
1000	12	0.94±0.19	1.00±0.18	+7
NAN-190(A)				
30	8	0.74±0.09	0.78±0.10	+6
100	8	0.68±0.10	0.76±0.10	+12
300	8	0.62±0.06	0.61±0.06	-1

* = $p \leq 0.01$ (Student's *t* test for paired data)

A) = higher doses were not tested because of the high toxicity and low solubility of this compound.

TABLE 7

Effects on cystometrogram in conscious rats.

Data represent the mean values ± S.E. (mmHg) of micturition pressure (MP), before and 15 min after i.v. injection of the compounds.

COMPOUND Dose µg/kg i.v.	No. of rats	MP before treatment	MP after treatment	% change
CONTROL vehicle	12	91.3± 9.2	87.9± 9.9	-4
Compound A				
100	8	93.0± 8.3	83.8± 8.7	-10
300	9	78.7± 5.8	70.0± 4.1	-11
1000	20	104.6± 6.4	91.0± 6.3*	-13
2000	10	101.8±10.9	81.5±14.1	-20
Flavoxate				
300	17	89.2±10.7	95.0±10.9	+7
1000	14	98.4±10.7	80.1±11.1	-12
3000	18	72.6± 9.3	75.2±9.5	+4
Oxybutynin				
100	13	95.2± 9.2	77.4±10.3*	-19
300	12	82.3± 8.7	50.5± 6.3*	-39
1000	12	110.9±10.2	29.6± 5.6*	-73
NAN-190(A)				
30	8	99.4±10.1	104.6± 9.7	+5
100	8	93.8±11.5	82.5± 9.2	-12
300	8	86.6±10.3	88.4±11.8	+2

* = $p \leq 0.01$ (Student's *t* test for paired data)

A) = higher doses were not tested because of the high toxicity and low solubility of this compound.

The administration of Compound A induced constant and significant increases of the BVC. Flavoxate (1000-3000 µg/kg) also induced increases in BVC, and the differences between basal and after treatment values were statistically significant (Table 6).

Oxybutynin was inactive on BVC (Table 6), but induced very consistent, significant and dose-related reductions of MP (the approximate ED_{50} value was 400 µg/kg), in contrast to Compound A and flavoxate which were inactive on this parameter (Table 7). NAN-190 was devoid of significant effects on both parameters up to the highest administrable dose of 300 µg/kg.

The effects of these compounds after oral administration were also tested. The results are shown in Tables 8 and 9 below.

TABLE 8

Effects on cystometrogram in conscious rats.

Data represent the mean values ± S.E. (ml) of bladder volume capacity (BVC), before and 1 hour after oral administration of the compounds.

COMPOUND Dose mg/kg p.o.	No. of rats	BVC before treatment	BVC after treatment	% change
CONTROL vehicle	11	0.64±0.10	0.73±0.13	+14
Compound A				
1	10	0.52±0.07	0.60±0.08	+15
3	10	0.67±0.07	0.91±0.10*	+35
10	10	0.54±0.06	0.73±0.10*	+37
Oxybutynin				
1	8	0.56±0.11	0.74±0.11*	+31
3	8	0.54±0.07	0.63±0.13	+18
10	8	0.55±0.08	0.70±0.11	+27
NAN-190				
10	10	0.54±0.08	0.46±0.07	-14
30	10	0.71±0.09	0.60±0.09	-15

* = $p \leq 0.01$ (Student's *t* test for paired data)

TABLE 9

Effects on cystometrogram in conscious rats.

Data represent the mean values ± S.E. (mmHg) of micturition pressure (MP), before and 1 hour after oral administration of the compounds.

Compound Dose mg/kg p.o.	No. of rats	MP before treatment	MP after treatment	% change
CONTROL vehicle	11	84.1±10.1	73.3±11.0	-13
Compound A				
1	10	96.0±8.4	93.7±7.2	-2
3	10	112.5±6.5	107.6±9.2	-4
10	10	90.2±7.1	86.6±7.6	-4
Oxybutynin				
1	8	92.1±13.3	77.3±9.8	-16
3	8	82.1±5.1	42.1±5.1*	-49
10	8	98.3±9.0	31.8±3.9*	-68
NAN-190				
10	10	106.1±10.4	90.8±12.5	-14
30	10	105.1±10.5	95.8±15.3	-9

* = p ±0.01 (Student's *t* test for paired data)

Compound A produced a significant increase of BVC after oral administration of 3 mg/kg, and no changes in MP values were detected. Oxybutynin caused a significant increase of the BVC after oral administration at the lowest utilized dose (1 mg/kg), and produced a dose-related reduction of the MP values that was consistent and significant with 3 and 10 mg/kg dose-levels (approximate ED_{50} value was 4 mg/kg). NAN-190 was inactive after oral administration at doses up to 30 mg/kg, a dose 10-fold higher than the minimal effective dose of Compound A.

These results were consistent with those obtained in anaesthetized rats as described in Example 8 above. Compound A was found to be active in increasing the BVC without affecting bladder contractility (MP), in contrast to oxybutynin. Compound A was also found to be active after both i.v. and oral administration, in contrast to NAN-190 which was inactive after i.v. or oral administration of doses up to 10-fold higher than those used for Compound A.

The above results show that compounds endowed with antagonistic activity at pre- and post-synaptic 5-HT_{1A} receptors and devoid of significant affinity for the α_1-adrenergic receptors are unexpectedly endowed with a potent pharmacological activity on the lower urinary tract. In particular, these compounds are able to inhibit the micturition reflex and to increase the period between micturition without impairing the capability of detrusor to have effective voidings once the micturition threshold has been reached. This is important since the drugs currently used for treatment of urinary incontinence (mainly anticholinergics) decrease efficiency of micturition, as shown for oxybutynin in Example 9, when the micturition pressure is reduced, and causes an increase of residual volume, due to compromission of bladder contractile force.

CLAIMS

1. Use of a compound which

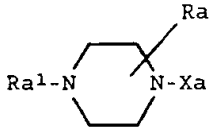
(a) binds to a 5-HT_{1A} receptor with an affinity of at least 10^{-7} M,

(b) binds to a 5-HT_{1A} receptor with an affinity at least 50-fold stronger than the affinity with which the compound binds to an α_1-adrenergic receptor, and

(c) exhibits 5-HT_{1A} receptor antagonist activity on both pre-synaptic and post-synaptic 5-HT_{1A} receptors,

or of a stereoisomer, hydrate, solvate or pharmaceutically acceptable salt of such a compound, for the preparation of a medicament for the treatment of lower urinary tract disorders in mammals.

2. Use according to claim 1 of a compound having the general formula I



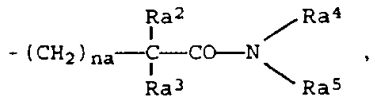
(I)

wherein

Ra represents a hydrogen atom or a lower alkyl group;

Ra^1 represents an aryl, nitrogen containing heteroaryl or bicyclic heteroaryl group;

Xa represents one of the groups

(Aa) 

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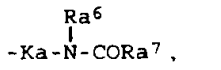
(Ba) 

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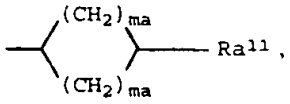
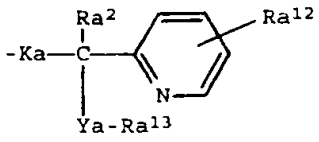
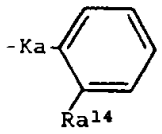
(Ca) 

,

(Da) 

and

(Ea)  ;

na is 1 or 2;

ma is 1, 2 or 3;

Ra^2 represents a hydrogen atom or a lower alkyl group;

Ra^3 represents an aryl or aryl(lower)alkyl group;

Ra^4 represents a hydrogen atom or a C_1-C_3 alkyl group;

Ra^5 represents a hydrogen atom or a C_1-C_3 alkyl, C_3-C_{12} cycloalkyl or cycloalkyl(lower)alkyl group; or Ra^4 and Ra^5 together with the nitrogen atom to which they are attached represent a 1-azetidinyl, 1-pyrrolidinyl, piperidino, 1-perhydroazepinyl, morpholino, or 1-piperazinyl group, each optionally substituted by a lower alkyl, aryl or aryl(lower)alkyl group;

Ra^6 represents a monocyclic or bicyclic heteroaryl group;

Ra^7 represents a hydrogen atom, a lower alkyl, cycloalkyl, cycloalkenyl, cycloalkyl(lower)alkyl, aryl, aryl(lower)alkyl, heteroaryl or heteroaryl(lower)alkyl group, or a group $-NRa^8Ra^9$ or ORa^{10};

Ra^8 represents a hydrogen atom or a lower alkyl, aryl or aryl(lower)alkyl group;

Ra^9 represents a hydrogen atom or a lower alkyl, -CO-(lower)alkyl, aryl, -CO-aryl, aryl(lower)alkyl, cycloalkyl or cycloalkyl(lower)alkyl group; or Ra^8 and Ra^9 together with the nitrogen atom to which they are attached represent a saturated heterocyclic group which may contain an additional heteroatom;

Ra^{10} represents a lower alkyl, cycloalkyl, cycloalkyl -(lower)alkyl, aryl, aryl(lower)alkyl, heteroaryl or heteroaryl(lower)alkyl group;

Ra^{11} represents an aryl or nitrogen containing heteroaryl group;

Ra^{12} represents a hydrogen atom or a lower alkyl group;

Ra^{13} represents a hydrogen atom or a C_1-C_3 alkyl, C_3-C_{12} cycloalkyl or cycloalkyl(lower)alkyl group;

Ra^{14} represents an aryl group;

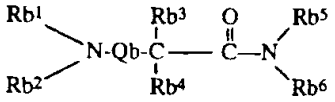
Ka represents a C_2-C_4 alkylene group optionally substituted by one or more lower alkyl groups; and

Ya represents a carbonyl, alkylene, hydroxyalkylene or hydroxycycloalkylene group or a group $-S(O)_{oa}$; where oa = 0 to 2.

3. Use according to claim 1 of 1-[N-cyclohexylcarbonyl-N-(2-pyridyl)--2-aminoethyl)-4-(2-methoxyphenyl)-piperazine.

4. Use according to claim 1 of 1-[N-cyclohexylcarbonyl-N-(2-pyridyl)--2-aminoethyl)-4-(4-indolyl)-piperazine.

5. Use according to claim 1 of a compound having the general formula II



(II)

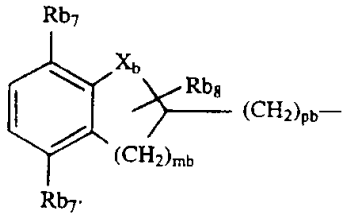
wherein

Qb represents a C_1-C_3 alkylene group, optionally substituted by one or more lower alkyl groups;

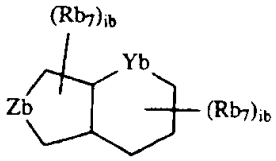
Rb^1 represents a hydrogen atom or a lower alkyl group;

Rb^2 represents one of the groups

(Ab)

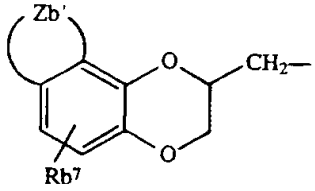


(Bb)



(Cb) Rb^9-CH_2CH_2- ,

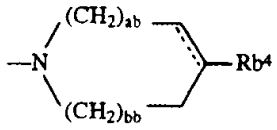
(Db)



(Eb) Rb^{10}-O-$CH_2CH(OH)CH_2$- and

(Fb) Rb^{10}-O-CH_2CH_2-;

or Rb^1 and Rb^2 together with the nitrogen atom to which they are attached represent a group of the formula



Rb^3 represents a hydrogen atom or a lower alkyl group;

Rb^4 represents an aryl, bicyclic aryl or heteroaryl group;

Rb^5 represents a hydrogen atom or a lower alkyl group;

Rb^6 represents a hydrogen atom or a C_1.C_{10} alkyl, C_3.C_{12} cycloalkyl, cycloalkyl(lower)alkyl, aryl or aryl(lower)alkyl group;

or Rb^5 and Rb^6 together with the nitrogen atom to which they are attached represent a saturated heterocyclic group, optionally containing an additional hetero atom and optionally substituted by a halogen atom or a lower alkyl, aryl, aryl(lower)alkyl, lower alkoxy or halo(lower)alkyl group;

ab is 0 to 3 and bb is 0 to 3 but (ab + bb) is not more than 3;

---- represents an optional double bond which can be present provided that ab is at least 1;

Xb represents a group $-(CH_2)_{nb}-$, $-OCH_2-$ or $-SCH_2-$;

mb is 0 or 1, nb is 1 to 3, and pb is 0 or 1; provided that (mb + pb) is 1 and (mb + nb) is not more than 3;

Rb^7 represents a hydrogen or halogen atom, or a lower alkyl, (lower)alkylcarbonyl, lower alkoxy, (lower)alkoxycarbonyl, hydroxy, trifluoromethyl, carboxamido, nitro, cyano, amino, (lower)alkylamino or di(lower)alkylamino group;

$Rb^{7'}$ represents a hydrogen or halogen atom; with the proviso that when Xb represents a group $-OCH_2-$ or $-SCH_2-$ then $Rb^{7'}$ represents a hydrogen atom;

Rb^8 represents a hydrogen atom or a lower alkyl group;

ib = 0, 1 or 2; jb = 0, 1 or 2;

Yb represents an oxygen or sulphur atom or a methylene group;

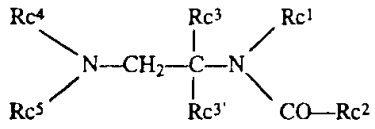
Zb represents the atoms necessary to form a heteroaromatic ring having from 5 to 7 carbon atoms fused to the non-aromatic ring containing the Yb group;

Rb^9 represents a monocyclic or bicyclic heteroaryl group;

Zb' represents either a pair of hydrogen atoms or the atoms necessary to form an aromatic or heteroaromatic ring fused to the benzodioxanyl group; and

Rb^{10} represents a monocyclic or bicyclic aryl or bicyclic heteroaryl group.

6. Use according to claim 1 of a compound having the general formula III



(III)

wherein

Rc^1 represents a heteroaryl or bicyclic heteroaryl group;

Rc^2 represents a cycloalkyl group;

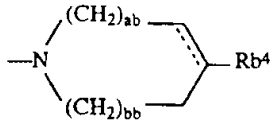
Rc^3 represents a hydrogen atom or a lower alkyl group;

$Rc^{3'}$ represents a hydrogen atom or a lower alkyl group;

Rc^4 represents a hydrogen atom or a lower alkyl group; and

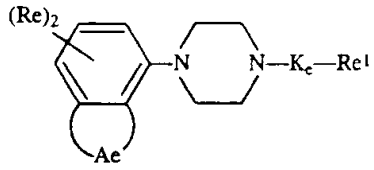
Rc^5 represents one of the groups (Ab), (Bb), (Cb), (Db), (Eb) and (Fb) as defined in claim 5,

or Rc^4 and Rc^5 together with the nitrogen atom to which they are attached represent a group of the formula



wherein ab, bb, Rb^4 and ---- are as defined in claim 5.

7. Use according to claim 1 of a compound having the general formula IV



(IV)

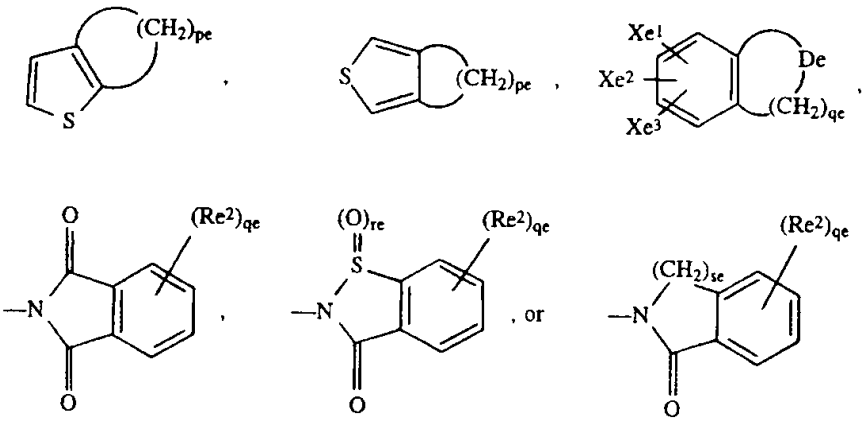
wherein

Ae represents a group -OCH=CH-, $-OCH_2CH_2-$, $-OCH_2O-$, $-OCH_2CH_2O-$ or -OCOCH=CH-;

each Re independently represents a hydrogen or halogen atom or an alkyl, hydroxy, alkoxy, trifluoromethyl or cyano group;

Ke represents a C_1-C_8 linear or branched alkylene group, optionally substituted by an aryl or heteroaryl group;

Re^1 represents a phenyl, thienyl, naphthyl or benzothiophenyl group, or a group of the formula



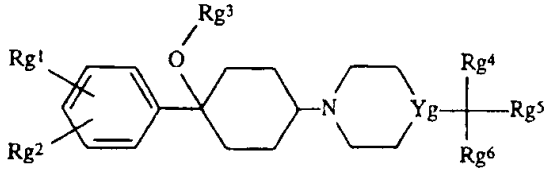
wherein pe is 3 or 4; qe is 0 to 3; re is 0 to 2; se is 1 or 2;

each Re^2 independently represents a halogen atom or an alkyl, hydroxy, alkoxy, trifluoromethyl or cyano group;

De represents a group -CH=CH- or $(CH_2)_{2-4}$; and

each of Xe^1, Xe^2 and Xe^3 independently represents a hydrogen atom or an alkyl, alkoxy, hydroxy, alkylthio, trifluoromethyl, nitro, amino or acetamido group, or two of Xe^1, Xe^2 and Xe^3 together represent a group $-OCH_2O-$ or $-OCH_2CH_2O-$.

8. Use according to claim 1 of a compound having the general formula V



(V)

wherein

each of Rg^1 and Rg^2 independently represents a hydrogen or halogen atom or a trifluoromethyl or C_1-C_4 alkoxy group;

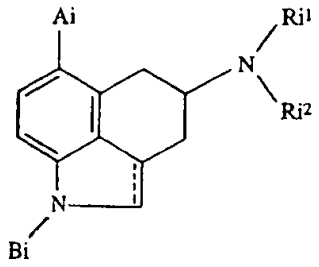
or Rg^1 and Rg^2, being on adjacent carbon atoms, together represent a group of formula $-O(CH_2)_{ig}O-$ wherein ig is from 1 to 3;

each of Rg^3, Rg^4 and Rg^5 independently represents a hydrogen atom or a C_1-C_4 alkyl group or a phenyl group;

Yg represents a nitrogen atom or a group CH; and

Rg^6 represents a heteroaryl, phenyl or substituted phenyl group.

9. Use according to claim 1 of a compound having the general formula VI



(VI)

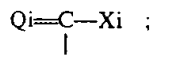
wherein

---- represents a single or a double bond;

Ri^1 represents a hydrogen atom, a C_1-C_4 alkyl, C_3-C_4 alkenyl, phenyl(C_1-C_4)alkyl or cyclopropylmethyl group, or a group $CORi^4$, $-(CH_2)_{ni}S(C_1$-$C_4)$alkyl or $-(CH_2)_{ni}C(O)NRi^9Ri^{10}$;

Ri^2 represents a hydrogen atom or a C_1-C_4 alkyl, C_3-C_4 alkenyl or cyclopropylmethyl group;

Ai represents a tetrazolyl or substituted tetrazolyl group, a heteroaryl group having 5 or 6 ring atoms of which from 1 to 3 may be oxygen, sulphur or nitrogen atoms, or a group



Bi represents a hydrogen atom, a C_1-C_4 alkyl group or an amino-blocking group;

Xi represents a hydrogen atom or a group $-ORi^3$, $-SRi^3$ or $-NRi^5Ri^6$;

Ri^3 represents a C_1.C_3 alkyl, substituted C_1.C_3 alkyl, aryl, substituted aryl, aryl(C_1.C_4)alkyl, substituted aryl(C_1.C_4)alkyl or C_3.C_7 cycloalkyl group;

Ri^4 represents a hydrogen atom or a C_1-C_4 alkyl, C_1-C_4 haloalkyl, C_1-C_4 alkoxy or phenyl group;

each of Ri^5 and Ri^6 independently represents a hydrogen atom or a C_1-C_4 alkyl, phenyl(C_1-C_4)alkyl or phenyl group;

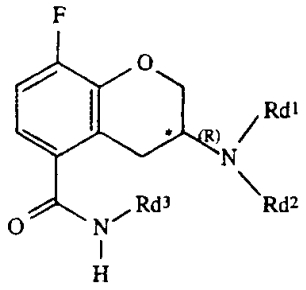
or Ri^5 and Ri^6 together with the nitrogen atom to which they are attached represent a C_3-C_5 heterocyclic ring;

each of Ri^9 and Ri^{10} independently represents a hydrogen atom or a C_1-C_4 alkyl or C_5-C_8 cycloalkyl group;

ni is 1 to 4; and

Qi represents an oxygen or sulphur atom.

10. Use according to claim 1 of a compound having the general formula VII



(VII)

wherein

Rd^1 represents an n-propyl or cyclobutyl group;

Rd^2 represents an isopropyl, t-butyl, cyclobutyl, cyclopentyl or cyclohexyl group; and

Rd^3 represents a hydrogen atom or a methyl group.

Fig.1.

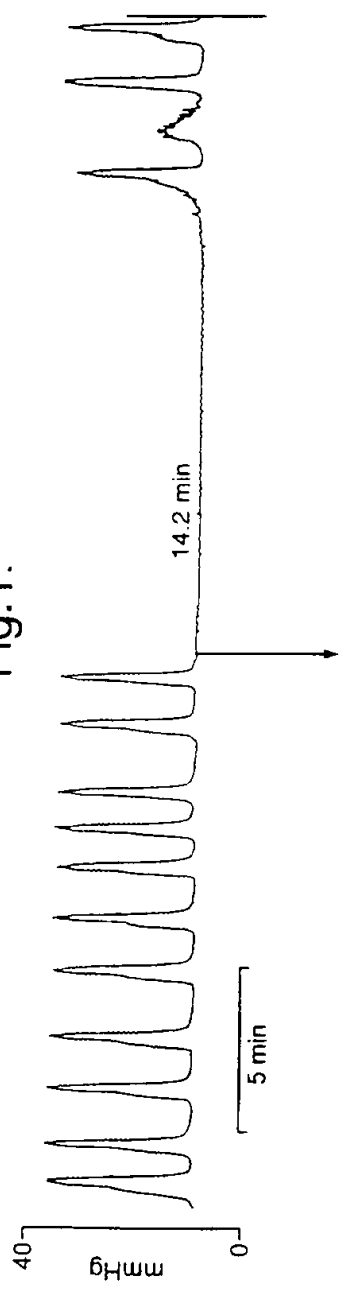


Fig. 1 - Typical tracing showing the effect of Compound A on volume-induced contractions of anaesthetized rats. In the basal period (15 min before the arrow) 9 peaks were recorded. After the i.v. administration of 300 μg/kg of Compound A (at the arrow), 14.2 min of bladder quiescence was observed (disappearance time; no contractions). No change in the height of the peaks was observed.

Fig.2.

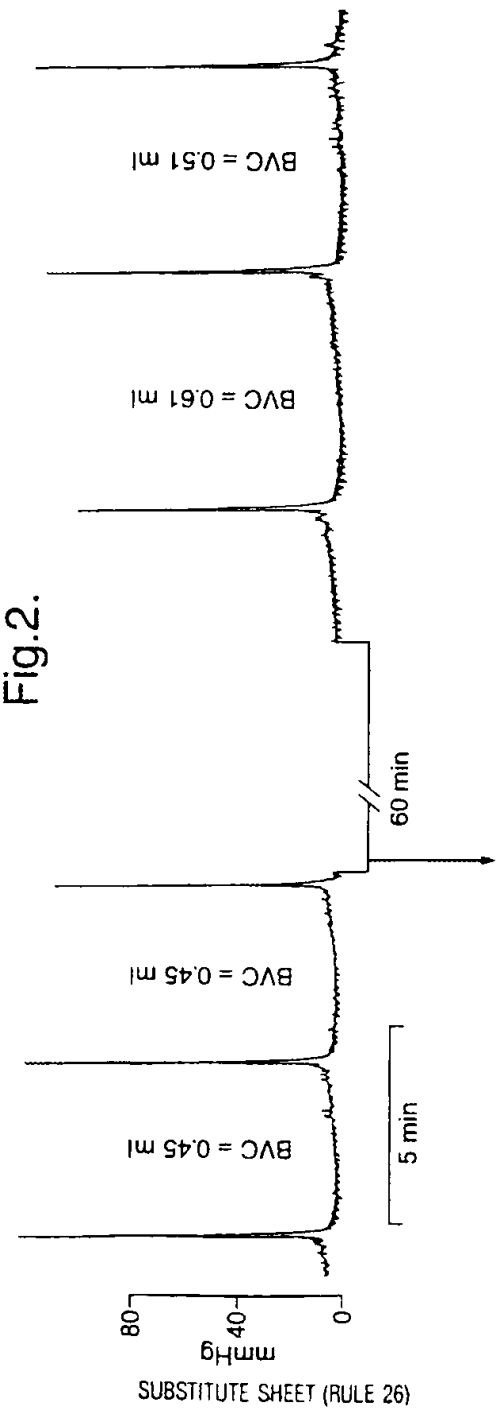


Fig. 2 - Typical tracing showing the effect of Compound A on cystometrographic parameters in conscious rats. Before treatment, two cystometrograms with the same bladder volume capacity (BVC) were recorded. Cystometry was then stopped and the animal was orally treated with 3 mg/kg of Compound A. Cystometrographic recording performed 60 min after the treatment gave two cystometrograms with BVC values of 0.61 and 0.51 ml (35.6 and 13.3% increase, respectively). No substantial changes in micturition pressure were recorded.

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/EP 97/00897

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/495

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 558 245 A (RECORDATI SA) 1 September 1993 see page 6; claims	1,10
P,X	WO 96 05817 A (MEDINNOVA) 29 February 1996 see the whole document	1-10
A	J.PHARMACOL.EXP.THER, vol. 262, no. 1, 1992, pages 181-189, XP000675356 A. LECCI ET AL.: "Involvement of 5-hydroxytryptamine 1A receptors in the modulation of micturition reflexes in the anaesthetized rat." cited in the application	
A	EP 0 512 755 A (WYETH) 11 November 1992	

☐ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
24 June 1997	03.07.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Klaver, T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/00897

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1,2,5-9
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
In view of the large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and compounds mentioned in the examples of the description.
SEE ANNEX

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. al Application No
PCT/EP 97/00897

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